Observation of GFP-fusion proteins in fixed cells[a]

Equipment and reagents

- 22x22 mm coverslips
- Paraformaldehyde (granular, Electron Microscopy Sciences)
- Microscopy coverslides
- Mounting medium (Molecular Probes)
- Filterpaper

Method

1. Transfect cells with GFP fusion protein of interest and seed the cells on sterile coverslips.
2. Grow cells for desired length of time, typically 16-24 hours.
3. Fix cells in 2% formaldehyde in PBS, pH 7.4 for 15 min. at room temperature[b]
4. Quickly rinse cells in 4 ml PBS, pH 7.4 and wash the coverslips in 2 ml PBS, pH 7.4, 2 X 5 min. at room temperature.
5. Place 10 ul of mounting medium on a glass microscopy slide. Remove excessive PBS by blotting the edges of the coverslip gently against a piece of filter paper.
6. Invert the coverslip cells facing down onto the mounting medium.
7. Remove excessive mounting medium by holding two pieces of filterpaper against two opposing edges of the coverslip until no more medium is drawn up by the filterpapers.

[a]This protocol applies to all versions of GFP, CFP (cyan), YFP (yellow), BFP (blue) and dsRed (red)

[b]Do not use methanol or acetic acid fixation as organic solvents destroy the autofluorescent properties of GFP.

- Visualization of GFP fusion proteins in fixed cells is compatible with detection of proteins by indirect immunofluorescence. After step 4, perform a normal indirect immunofluorescence.